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水蕨卵膜的形成及其超微结构的观察*

曹建国, 杨耐英, 王全喜
(上海师范大学 生命与环境科学学院, 上海 200234)

摘要: 蕨类植物成熟卵的周围有一层卵膜, 但其细微结构和形成过程仍不清楚, 本研究应用透射电镜技术对水蕨 (*Ceratopteris thalictroides*) 卵细胞发育过程中卵膜的形成及超微结构进行了观察。结果表明水蕨卵细胞在发育中期开始形成卵膜, 卵上方的卵膜十分显著, 是由多层嗜锇性内质网片层附着于质膜内表面形成的, 成熟时卵上方的卵膜中心部分厚, 向边缘逐渐变薄, 在嗜锇性片层之间填充有嗜锇性物质。比较而言, 卵下方及侧面的卵膜薄, 由两层紧密连接的嗜锇性膜构成。首次阐明了蕨类植物卵膜形成的超微结构, 并对卵膜的一些功能进行了探讨。

关键词: 蕨; 水蕨; 卵膜; 超微结构
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Observations on the Formation and Ultrastructure of the Egg Membrane in the fern *Ceratopteris thalictroides* (Parkeriaceae)

CAO Jian-Guo, YANG Nai-Ying, WANG Quan-Xi
(*College of Life and Environment Science, Shanghai Normal University*, Shanghai 200234, China)

Abstract: The mature egg of ferns possesses a layer of egg membrane in the periphery. However, the fine trastructure and the formation of the egg membrane in the egg of ferns are still unclear. The present paper described the formation and the ultrastructure of the egg membrane in the egg of the fern *Ceratopteris thalictroides* using transmission electron microscopy (TEM). The results show that the egg membrane begins to form at the stage of the maturing egg. The egg membrane in the upper side of the egg is prominent. It is formed by attaching of osmiophilic sheets of endoplasmic reticulum to the inner surface of the plasmalemma. When mature the egg membrane in the upper side of the egg is much thick in the central region and it becomes thinner gradually towards the margin. Some osmiophilic materials are filled in the spaces between the sheets of endoplasmic reticulum. On the contrary, the egg membrane in the lower side of the egg is thin. It is composed of two osmiophilic membranes which are associated with each other closely. The formation and the ultrastructure of the egg membrane are described for the first time. Some functions of the egg membrane are also discussed.

Key words: Fern; *Ceratopteris thalictroides*; Egg membrane; Ultrastructure

Bell and Mühlethaler (1962) found an egg membrane in the mature egg of *Pteridium aquilinum* (L.) Kuhn. Similar membrane has been detected in eggs of many ferns including *Dryopteris filix-mass* (Menke and Fricke, 1964), *Histiopteris incise* (Bell, 1980), *Todea* (Bell, 1986), *Osmunda cinnamomea* var. *asiatica* (Bao *et al.*, 2003) and *Dryopteris crassirhizoma* (Bao *et al.*, 2005). The egg membrane was considered to be derived from the degenerated organelles or the vesicles in the cytoplasm of the egg and formed out-

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作者简介: 曹建国 (1968-) 男, 副教授, 主要从事蕨类植物发育与生殖生物学研究。E-mail: cao101@shnu.edu.cn side the plasmalemma (Bell and Mühlethaler, 1962; Bell, 1986; Bao *et al.*, 2003; Bao *et al.*, 2005). It has been proved that the egg membrane around the egg of *P. aquilinum* is lipid in nature (Cave and Bell, 1974). However, the formation and the ultrastructure of the egg membrane in the egg of ferns are still unclear. In the present paper, the ultrastructure and the formation process of the egg membrane in the egg of the fern *Ceratopteris thalictroides* was observed by transmission electron microscope. Some new phenomena about the formation and the structure of egg membrane, different from the previous studies, are described.

1 Materials and methods

The spores of *C. thalictroides* (L.) Brongn. were collected from plant grown in the Botanical Garden of Shanghai Normal University. The culture of gametophytes were obtained as follow: The mature spore, soaked in sterilized water for 24 h in advance, was sterilized with 5% sodium hypochlorite solution for 5 minutes and then washed with sterilized water 3 times. The spores were sown in the culture medium of modified Knop's solution, solidified with 1.5% agar. The materials were cultured at the condition of about 25℃, fluorescent light (ca. 35 μmol·m^{-2}·s^{-1}) with 18 h illumination and 6 h night. After about 3 to 4 weeks later, the gametophyte containing archegonia was picked up and plunged into 3% glutaraldehyde in 0.1 mol/L phosphate buffer at room temperature for 6 - 12 h. The materials were subsequently washed with the same buffer for three times and fixed in osmic acid (2% aqueous solution) for overnight and then washed with the same buffer 3 times. After dehydration in a graded acetone series, the material was infiltrated with mixture of acetone and spurr's resin and then embedded in the pure resin. Specimens were thick sectioned firstly for the presence of the archegonium and then thin-sectioned with a diamond knife on an Ultracut-E ultramicrotome and mounted on grid. The thin sections were stained with uranyl acetate and lead citrate. All specimens were observed with H-600 electron microscope.

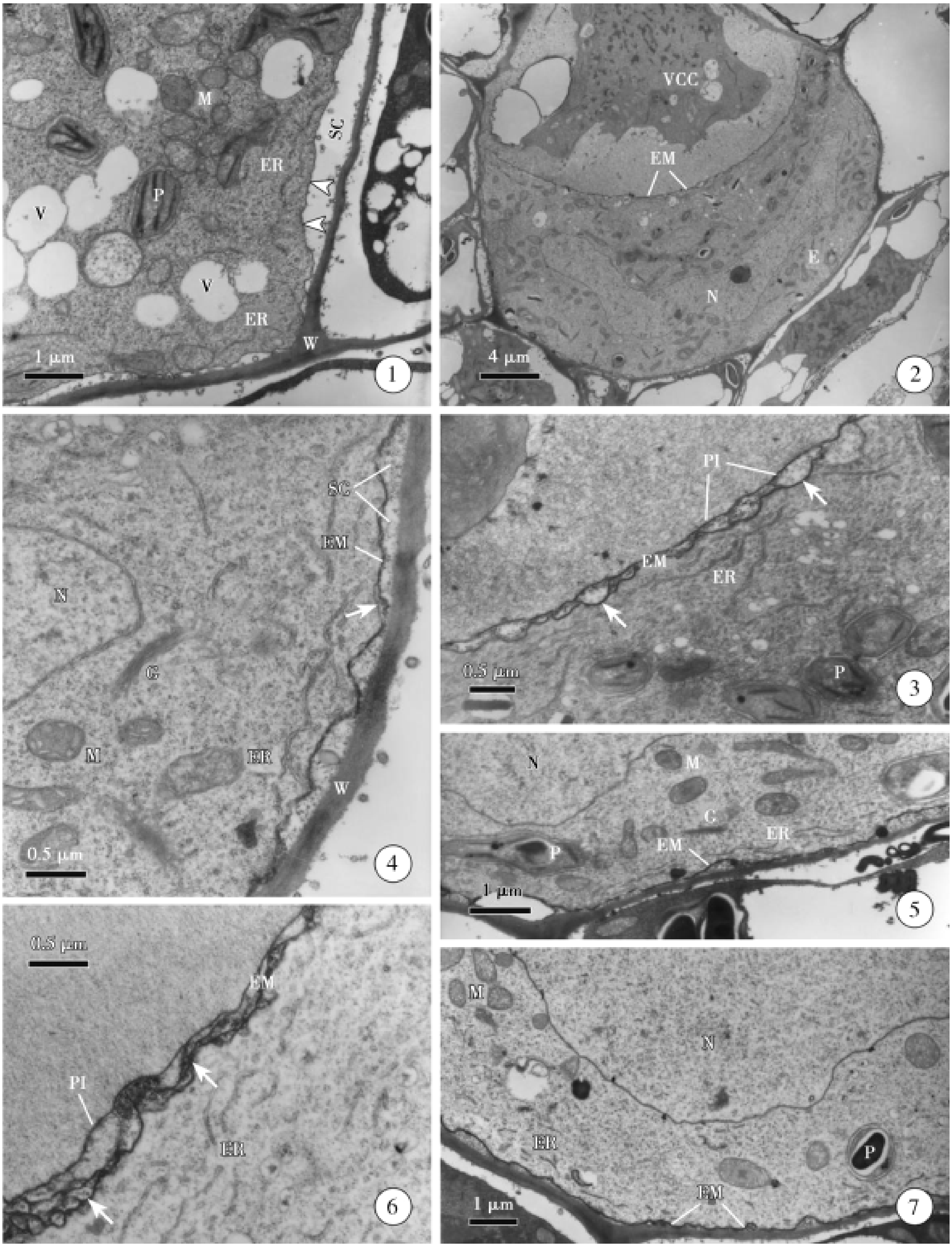
2 Results of observation

2.1 The development of egg and the formation of the egg membrane

The development of the egg of ferns is generally divided into 3 stages, i.e. the young egg, the maturing egg and the mature egg (Bell and Duckett, 1976). In the young egg of *C. thalictroides*, there are abundant organelles, including plastids, mitochondria, vesicles and endoplasmic reticula in the cytoplasm. These organelles are basically distributed through out the cytoplasm of the egg except the endoplasmic reticula, which are principally located at the periphery (fig.1). The plasmalemma of the egg can be identified clearly (fig.1, arrowhead). The cell wall between the egg and the adjacent cells is well defined and devoid of plasmodesmata. At the late stage of the young egg, a separate cavity (fig.1, SC) is formed between the plasmalemma of the egg and the wall of the archegonium as the eliminating of vesicles from the cytoplasm of the egg cell. No additional membrane was formed around the egg in this stage.

In the stage of the maturing egg, a striking feature of the egg of *C. thalictroides* is that a layer of osmiophilic membrane is formed around the egg. The osmiophilic membrane is not existed around the ventral and the neck canal cells. So the membrane is named as an egg membrane (fig.2, EM). The egg membrane in the upper side of the egg is prominent (figs.3 - 5). Most likely the endoplasmic reticula are involved in the formation of the egg membrane in this stage. Firstly, many sheets of endoplasmic reticulum in the egg cytoplasm lie closely and parallel to the plasmalemma (fig.3). When a sheet of endoplasmic reticulum (fig.3, arrow) is attached to the inner surface of the plasmalemma, both the sheet of endoplasmic reticulum and the plasmalemma, become osmiophilic. The two osmiophilic membranes form the egg membrane (fig.3, EM). At the side and the lower part of the egg, although some endoplasmic reticula lie closely to the inner surface of the egg, no additional sheet of endoplasmic reticulum was seen to be attached to the plasmalemma (figs.4, 5). The osmiophilic membrane, covering the lower surface of the egg, seems to be a double-layered membrane, occasionally two membranes can be detected in places (fig.4, arrow).

With the developing of the egg cell, more sheets of endoplasmic reticulum are attached to the inner surface of the upper egg membrane. As soon as the new sheets of endoplasmic reticulum are attached to old ones, they also become osmiophilic (fig.6, arrow). In addition, the osmiophilic sheets, constituting the egg



Figs. 1-7 The formation of the egg membrane in the egg of *Ceratopteris thalictroides*

1. A part of young egg contains plenty of organelles including mitochondria (M), plastid (P), vesicles (V) and endoplasmic reticula (ER), which are principally located in the periphery of the egg. A separate cavity (SC) formed between the plasmalemma (arrowhead) and the wall (W) of the archegonium; 2. A maturing egg (E) is surrounded by a layer of osmiophilic egg membrane (EM). The ventral canal cell (VCC) is degenerating; 3. Magnification of figure 2 showing the egg membrane (EM) in the upper side of the egg. The egg membrane formed by attaching of a sheet of endoplasmic reticulum (arrow) to the plasmalemma (Pl); 4. The same egg cell with figure 2 showing the egg membrane (EM) and endoplasmic reticula in side of the egg; 5. The same egg cell with figure 2 showing the egg membrane (EM) and endoplasmic reticula in lower side of the egg; 6. The egg membrane (EM) develops further by attaching of more sheets of endoplasmic reticulum (arrow) to the inner surface of the egg membrane; 7. The lower part of the same egg cell with figure 6 showing the egg membrane (EM) in the lower of the egg unchanged. Abbreviation: N, nucleus; G, Golgi body

membrane, are interconnected with each other. Finally, a multilayered osmiophilic egg membrane is formed inside the plasmalemma (fig.6). In the cytoplasm, some redundant endoplasmic reticula are also existed (fig.6). By contrast, the egg membrane in the side and lower part of the egg remain almost unchanged (fig.7).

In the late stage of the maturing egg, another conspicuous feature of the egg is the occurrence of numerous small vesicles containing the strongly osmiophilic materials. These vesicles, with a diameter about 0.3 - 0.5 μm, are principally located in the cytoplasm above the cup-liked nucleus (Figs.8, 9, Vo). The vesicles are undoubtedly involved in the formation of the egg membrane in the upper side of the egg. The vesicles move firstly to the inner surface of the upper egg membrane and soon these vesicles are attached to the egg membrane (fig.9, Vo). Occasionally, the fusion of vesicles with the egg membrane is observed clearly. At the same time, the osmiophilic materials within the vesicles begin to decondense into small granules, which are undoubtedly incorporated into the egg membrane (fig.10). Thus, the egg membrane completes its formation. The maturing egg also contains plenty of organelles, including the degenerated plastids, the mitochondria and Golgi bodies (figs.3 - 5).

2.2 The structure of the egg membrane in the mature egg

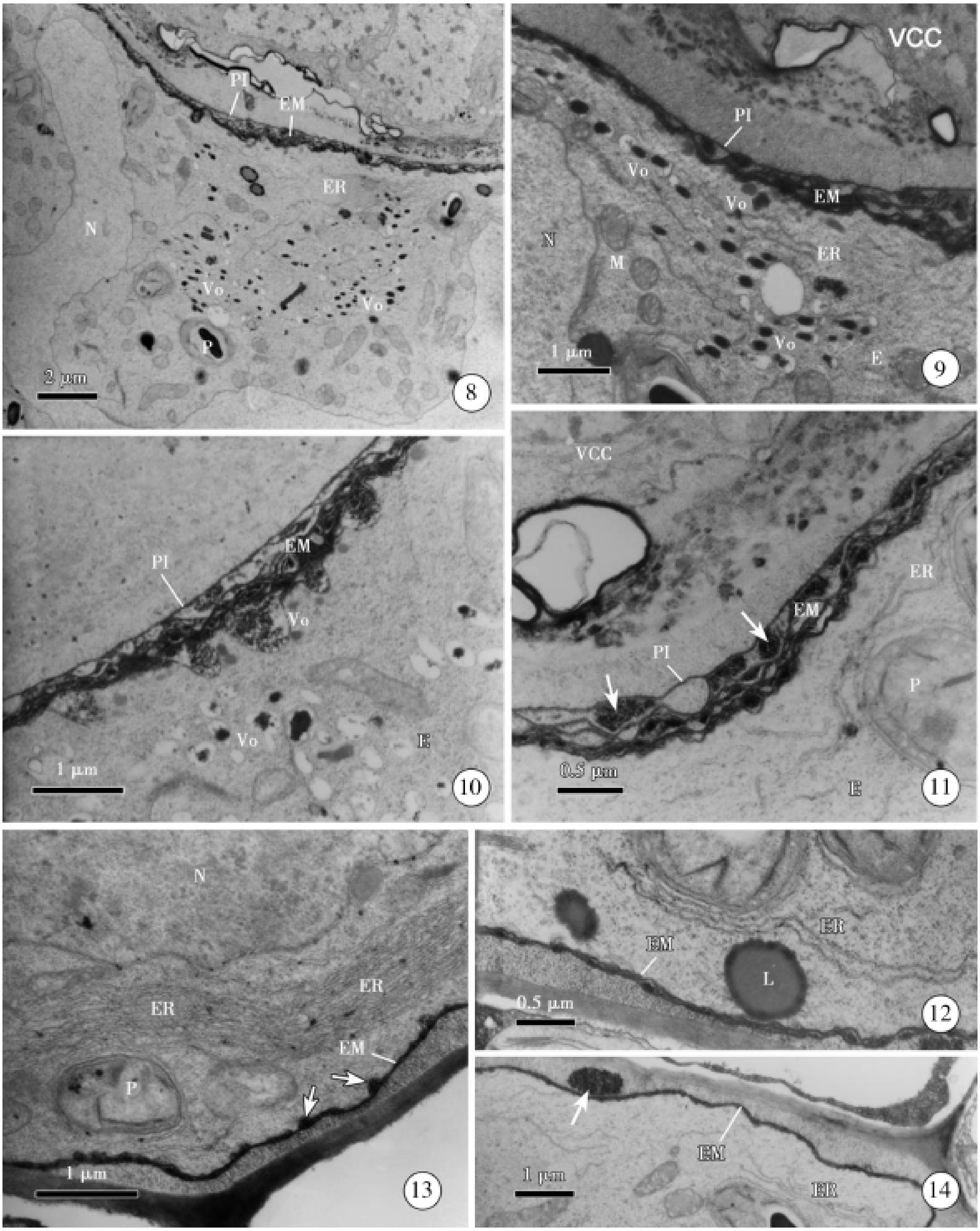
In the mature stage, the egg completes the formation of the egg membrane. No sheets of endoplasmic reticulum are attached to the egg membrane. A conspicuous feature of the egg is that the endoplasmic reticula, previously lying closely to the periphery of the egg, now form stacks in the cytoplasm (figs.11 - 14). The structure of the egg membrane in the upper side of the egg can be identified clearly. The outmost layer of the egg membrane is undoubtedly the plasmalemma which is much smoother than the osmiophilic sheets beneath it (fig.11). The osmiophilic sheets of endoplasmic reticulum beneath the plasmalemma, with an undulated and rough shape, are interconnected with each other. The spaces between the sheets are filled with osmiophilic materials, which are condensed again after being incorporated into the egg membrane (fig.11, arrow). Another feature of the mature egg is that the thickness of the egg membrane in the upper side of the egg is not equal. In the central portion, the thickness of the egg membrane reaches or exceeds 0.5 μm and it becomes thinner gradually towards the margin (figs.8, 12).

The egg membrane in the side and lower part of the egg has a relatively simple structure. It is a layer of osmiophilic membrane at first sight. But at high magnification, two layers of osmiophilic membrane can be detected clearly (fig.12). The thickness of the egg membrane increases to about 70 - 80 nm. Occasionally, small osmiophilic granules are seen being attached to the egg membrane (fig.13, arrow). In the mature egg, some large lumps of osmiophilic materials are often seen in the cavity between the egg membrane and the wall of the archegonium (fig.14, arrow).

3 Discussion

3.1 The formation and development of the egg membrane

The mature egg of *C. thalictroides* possesses a layer of egg membrane, which has been proved to be existed in egg of other homosporous ferns including *Pteridium aquilinum* (Duckett and Bell, 1972; Cave and Bell, 1974), *Histiopteris incise* (Bell, 1980), *Osmunda cinnamomea* var. *asiatica* (Bao *et al.*, 2003), *Dryopteris crassirhizoma* (Bao *et al.*, 2005). The egg membrane is believed to be formed by the deposition of materials which are derived from the degenerated organelles or vesicles in the cytoplasm of the young egg on the outside of the plasmalemma. Although the young egg of *C. thalictroides* possesses plenty of vesicles, no osmiophilic materials within the vesicles are observed in the young egg. There are also no evidence that the vesicles are derived from the degenerated mitochondria or plastids. So we considered that the egg membrane is not formed by the osmiophilic materials within the vesicles in the young egg. In this study, we described the detailed formation processes of the egg membrane in the upper side of the egg. It is proved that the egg membrane, especially the upper side, is formed directly by sheets of the endoplasmic reticulum. As early as in the young egg, the sheet of endoplasmic reticulum is observed to lie closely to the periphery of the egg. As soon as the sheet of endoplasmic reticulum is



Figs. 8-14 The formation of the egg membrane in the egg of *Ceratopteris thalictroides*

8. Numerous vesicles containing strongly osmiophilic materials (Vo) occurring in the cytoplasm at the late stage of the maturing egg; 9. Magnification of the same egg cell with figure 8, showing the vesicles containing osmiophilic materials (Vo) move to the egg membrane (EM); 10. The vesicles containing osmiophilic materials (Vo) are fused with the egg membrane; 11. The mature egg membrane in the upper side of the egg, consisting of membranous sheets and osmiophilic materials (arrow); 12. The egg membrane (EM) in the side of the mature egg, two layers of membrane can be detected clearly; 13. The small osmiophilic material granules are attached to the egg membrane (arrow). Stacks of endoplasmic reticula (ER) are often seen in the cytoplasm of the mature egg; 14. Some lumps of osmiophilic materials (arrow) are often seen in the cavity between the egg membrane and the wall of the archegonium in the mature egg. **Abbreviation:** E, egg; M, mitochondrion; N, nucleus; P, plastid; Pl, plasmalemma; VCC, ventral canal cell

attached to the inner surface of plasmalemma, both of them become osmiophilic and form the egg membrane. In succession, more sheets of endoplasmic reticulum will be attached to the inner surface of the egg membrane. Thus the membranous egg membrane is formed. After that, it is also demonstrated that the new formed vesicles containing the strongly osmiophilic materials in the cytoplasm of the egg are involved in the formation of the egg membrane. The distinct fusion of the vesicles with the egg membrane suggested the osmiophilic materials within the vesicles are incorporated into the egg membrane (fig.10). However, the origin of the new formed vesicle is still uncertain. We propose that the occurrence of the vesicles may be relative to the abundant endoplasmic reticulum in the cytoplasm of the egg.

3.2 The structure and function of the egg membrane

The detail structure of the egg membrane is still uncertain in the ferns so far reported (Bell and Duckett, 1976; Bell, 1980; Bao *et al.*, 2003; Bao *et al.*, 2005). In present investigation, the fine structure of the upper egg membrane in the fern *C. thalictroides* is described for the first time. The detailed observations about the formation of the egg membrane suggested that the outmost layer of the upper egg membrane is undoubtedly the plasmalemma and beneath it are the osmiophilic sheets of endoplasmic reticulum. Here, it is suggested that the sheets beneath the plasmalemma are the fundamental skeleton of the egg membrane. After the membranous skeleton was formed, the osmiophilic materials within the vesicles are incorporated into the egg membrane. By contrast, the egg membrane in lower part of the egg is much simple, only in high magnification, the two osmiophilic membranes can be detected. What role the special egg membrane plays is much interesting. It is known that the egg membrane around the egg of *P. aquilinum* is made up of lipid material, which may be used as a barrier to protect the egg from the ingress of metabolites or "informational" molecules from elsewhere in the gametophyte (Cave and Bell, 1974; Bell and Duckett, 1976). The egg membrane of the egg of *C. thalictroides* is much prominent. Its role may be the same with that in *P. aquilinum*. In addition, the distinct differences in morphology and structure of the egg membrane between the upper part and the lower part of the egg may mean the great differences in function of them. The thick upper egg membrane, exposed to the sperms, suggests that it may be used to prevent the sperms from entering the egg. The entrance of the sperm may be a pore, which is found recently in the egg membrane (unpublished). A further research will be conducted about the function of the egg membrane. Moreover, the osmiophilic lumps in the separate cavity are also encountered frequently in the mature egg, which may also be used to prevent the sperms from entering the egg in the lateral side.

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